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Review

Mesenchymal stem cell-derived exosomes as a new drug carrier for the treatment of spinal cord injury: A review

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ABSTRACT

Spinal cord injury (SCI) is a devastating traumatic disease seriously impairing the quality of life in patients. Expectations to allow the hopeless central nervous system to repair itself after injury are unfeasible. Developing new approaches to regenerate the central nervous system is still the priority. Exosomes derived from mesenchymal stem cells (MSC-Exo) have been proven to robustly quench the inflammatory response or oxidative stress and curb neuronal apoptosis and autophagy following SCI, which are the key processes to rescue damaged spinal cord neurons and restore their functions. Nonetheless, MSC-Exo in SCI received scant attention. In this review, we reviewed our previous work and other studies to summarize the roles of MSC-Exo in SCI and its underlying mechanisms. Furthermore, we also focus on the application of exosomes as drug carrier in SCI. In particular, it combs the advantages of exosomes as a drug carrier for SCI, imaging advantages, drug types, loading methods, etc., which provides the latest progress for exosomes in the treatment of SCI, especially drug carrier.

1. Introduction

Spinal cord injury (SCI) is a devastating neurological disease associated with serious disturbances in autonomic nervous system function, resulting in permanent neurological deficits and sharply diminished quality of life that make patients and their families feel hopeless.[1–3] It also leads to motor and sensory disorders and even life-threatening respiratory distress. The primary injury is caused by mechanical force and is temporary but creates a basic premise for the subsequent cascade of secondary pathological reactions[4], including neuronal apoptosis and inflammatory reactions that result in a toxic microenvironment, which is a major culprit in the loss of nerve functions[5,6]. However, protecting the transient primary injury is almost impossible, and suppressing a cascade of secondary injuries is still a treatment focus. Mesenchymal stem cells (MSCs) and MSC-derived exosomes (MSC-Exo) have been proven to exert a therapeutic potential in SCI.[7–9] In this review, we systematically reviewed experimental studies of MSC-Exo in the treatment of SCI over the last 4 years and summarized current techniques for MSC-Exo delivery and problems for SCI treatment. We highlighted the mechanisms responsible for exosomes' roles in structural and functional recovery in this malady for the proposal of modified and more effective exosome treatment strategies in meaningful clinical trials in the future.

2. Emerging approaches for SCI treatment

Current problems we face in the treatment of SCI (Fig. 1) incorporate neural apoptosis stimulated by the trauma[10] and very limited self-regenerative and proliferative functions of mature neurons[5,11–13]. Here are the 4 approaches currently used to repair the spinal cord (Fig. 1). The first 2 approaches involve the recovery of the microenvironment surrounding the injured spinal cord by inhibiting the inflammatory response, minimizing cytotoxicity, or relieving mechanical pressures restricting axonal growth[9,14,15] and identification and regulation of relevant signalings or molecules to trigger self-repair of damaged neurons and boost neural regeneration.[16–19] Moreover, transplantation of MSCs, which can self-renew and differentiate into neuronal cells, into the injured spinal cord[16,19–21], and cell-free therapy, such as injection of stem cell-derived exosomes or adenovirus-derived small interfering RNAs (siRNAs) into the injured sites.[1,22–24]

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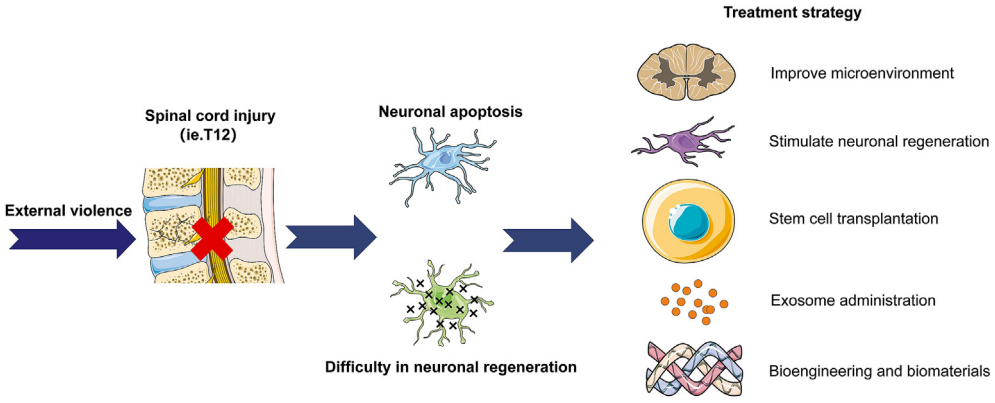

Fig. 1. Problems and solutions for treatment of spinal cord injury.

Recent advances in bioengineering, including biomaterials or genetic engineering, provide more options for patients excepting a better prognosis after treatment.[25–27] In the past decade, these techniques have shown significant improvements in axon regeneration, scar formation, nerve cell transplantation, examinations of inflammatory mediators, and most importantly, identification of spinal cord automaticity and molecular mechanisms for functional recovery after SCI.[28–30] Li et al.[31] combined with MSCs-Exo and hydrogel therapy have successfully promoted neurological recovery and urinary tissue protection in SCI rats. Kim et al.[32] also used engineering technology to incorporate magnetic iron oxide nanoparticles (IONP) into nanovesicles derived from human MSCs and used the navigation function of magnets and the drug-loading function of exosomes to carry more therapeutic growth factors to the SCI area.

3. Problems with the treatment of SCI

However, problems with a lack of highly repeatable and effective approaches in the personalized treatment of SCI are still present. Many *in vitro* results cannot be replicated in animal experiments, let alone clinical studies. Multitherapy combination strategies could be more beneficial, but maximal performances of every single therapy might not be warranted, which needs more and stronger evidence from animal experiments and clinical trials of combined therapies. Therefore, more promising individualized treatment schemes have been the focus of several previous and ongoing clinical trials and laboratory experiments.

4. Discovery and characterization of exosomes

Exosomes were discovered when researchers observed the formation of extracellular vesicles and their components in the membrane around the 1980s. In 1977, De Broe et al.[33] first reported segments of the plasma membrane of secretion in human duodenal fluid, the secretion of villous and tubular adenomas, and Hela cell culture medium (Fig. 2A). These plasma membrane fragments carry the same plasma membrane enzymes as the original cells and have the general characteristics of living cells. Trams et al.[34] described the vesicle structure of these fragments containing extracellular enzymes in 1981 and first proposed the term "exosomes". However, this study did not provide more information on their shedding from cell membranes due to the microscopy techniques of their time. In 1985, Pan et al.[35] first ascertained that such vesicles with a diameter of 50 nm were released outside the cell after the fusion of early endosomes with cell membrane mediated by the shedding of transferrin receptors, which undoubtedly improved the knowledge of exosome formation. This extracellular vesicle as we know it today is classified into 3 types: exosomes (< 150 nm in diameter), microvesicles (100 – 1000 nm), and apoptotic bodies (> 1000 nm), based on their size[36,37], which are characterized by different shapes and functions. Among them, exosomes have been a hotspot for drug delivery.

Sun et al.[7] found exosomes derived from human umbilical cord MSCs could improve locomotor function and attenuate inflammation in a murine contusion SCI model. The exosomes likely exerted effects by deactivating microglia and macrophages. Li et al.[9] engineered MSCs to overexpress microRNA (miR)-133b and showed the secreted exosomes containing miR-133b could stimulate neurite remodeling and functional improvement after SCI in rats. Huang et al.[38] demonstrated systemic delivery of MSC exosomes in rats after SCI reduced neuronal apoptosis, suppressed pro-inflammatory cytokines, and promoted angiogenesis and tissue sparing. Liu et al.[39] found bone marrow MSC (BMMSC)-Exo could suppress neurotoxic A1 reactive astrocyte activation after SCI through exosomal transfer of miR-133b. Fan et al.[40] developed an electroconductive hydrogel loaded with exosomes derived from adipose-derived MSCs. They found the exosome-hydrogel system could simultaneously regulate the immune microenvironment and enhance myelinated axon growth in a rat model of SCI, leading to significant locomotor recovery. The effects were mediated in part by exosomal miR-21-5p. Ren et al.[41] showed exosomes derived from milk fat globule-EGF-factor VIII overexpressing Schwann cells could switch macrophage/microglial polarization to an anti-inflammatory phenotype and promote axon regeneration and functional recovery after SCI in rats. The immunomodulatory effects involved suppressors of cytokine signaling mediated inhibition of signal transducer and activator of transcription 3. Xiong et al.[42] demonstrated that regulatory T cell-derived exosomes delivering miR-709 could attenuate neuronal pyroptosis and microglial activation while promoting motor function after SCI in mice. Lu et al.[43] used exosomes enriched with netrin-1 mRNA to reduce inflammation and pyroptosis and stimulate axonal regrowth following SCI through microRNA (miRNA)-mediated suppression of downstream pathways. Taken together, these recent studies highlight the potential of exosomes to treat SCI.

5. Biogenesis and biological functions of exosomes

Exosomes are membrane vesicles generated by all cells and present in human and mammalian blood, saliva, urine, and milk, typically 30 – 130 nm in diameter.[44–46] Exosome formation, though complex, can be summarized into 2 steps (Fig. 2B). In the first step, after secretory cells receive intracellular and extracellular

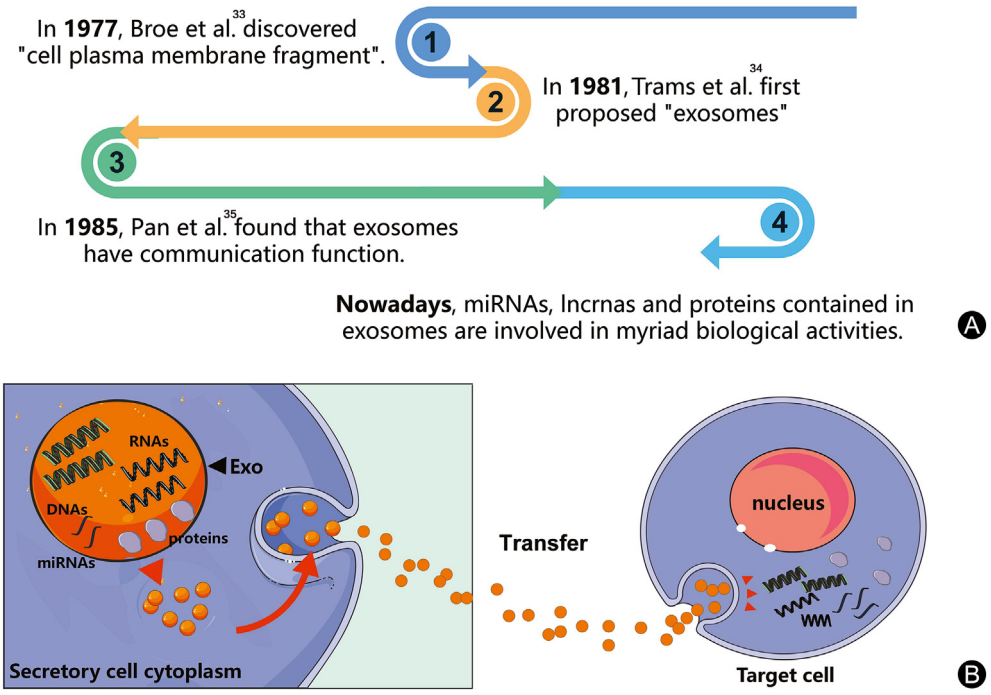

Fig. 2. Discovery and biogenesis of exosomes. (A) Discovery of exosomes. (B) Exosomes regulate intercellular communication.

signals, part of the plasma membrane invaginates to form early endosomes that subsequently form multi-vesicular bodies in the cytoplasm. These multi-vesicular bodies are degraded by lysosomes, released from cells via activation of cytoskeletal proteins and kinesins, and form exosomes outside of the cell to deliver molecules (proteins, lipids, and RNAs) to target cells.[47–49] Thus, exosomes act as shuttles that enhance communication between cells.[48,49]

The exosome-mediated transfer is the most prominent biological feature of intercellular communication, which delivers signal molecules to target cells via receptor-mediated phagocytosis or pinocytosis, a significant way for cells to take materials from exosomes.[50] These are direct exosome membrane fusion with target cells.[51] Also, exosomes can recognize and activate target cells via generating signaling after ligands on the exosome surface bind to receptors on the cell membrane. For example, mitochondrial double-stranded RNA in exosomes secreted by hepatocytes can enhance the production of interleukin (IL)-17A production through toll-like receptor 3 in Kupffer cells.[52]

Upon direct and indirect connections, exosomes transfer signals to trigger target cells, leading to changes in cell proliferation, differentiation, immune response, and apoptosis.[36]

6. Treatment of SCI using MSC-Exo

6.1. Immunomodulation: MSC-Exo suppress inflammation after SCI

As shown in Fig. 3, the inflammatory response is the culprit in producing secondary nerve injuries to expand the damaged area after SCI[53–55], as mentioned above, exacerbating blood-spinal cord and vascular endothelial barrier leakage to allow peripheral lymphocytes, macrophages, and neutrophils to infiltrate into the damaged sites to release more inflammatory mediators. Undoubtedly, an inflammatory cascade can lead to exceptional increases in local oxidative stress, autophagy, and apoptosis.[22,56]

Macrophages are innate immune cells crucial in the inflammatory response, consisting of cytotoxic or pro-inflammatory M1 and anti-inflammatory M2 subtypes or homeostatic.[57,58] Ding et al.[59] found that in the central nervous system (CNS) injury disease rats model, MSC-Exo could convert M1 macrophages into M2 macrophages, thus significantly upregulating the expressions of the anti-inflammatory factors IL-10 and TGF-β1. Liu et al.[60] found that miR-216a-5p in MSC-Exo could mediate toll-like receptor 4 (TLR4)/nuclear factor kappa B (NF-κB)/phosphoinositide 3-kinases (PI3K)/protein kinase B (Akt) signaling cascade is involved in the transformation of microglia M1 phenotype to M2 phenotype.

Shiue et al.[61] demonstrated that intrathecal injection of MSC-Exo markedly decreased tumor necrosis factor-α (TNF-α) and IL-1β levels in injured tissues of rats with neuropathic pain caused by L5/6 dorsal root ganglion injury. MiRNA-abundant MSC-Exo can target key components of inflammatory signaling pathways to terminate the signaling, and thereby curb the generation of inflammatory mediators.[62] Sun et al.[63] reported that miR-27b suppressed Jumonji domain-containing protein D3, and NF-κB expressions, thus inhibiting gene transcriptions of pro-inflammatory TNF-α, IL-1β, and IL-6.

Therefore, MSC-Exo carrying anti-inflammatory factors is an option to forestall secondary nerve damage in SCI.

6.2. Antioxidant effects: MSC-Exo alleviate oxidative stress

Oxidative stress is a state of imbalance between aerobic metabolic and antioxidant systems. The reactive oxygen species and reactive nitrogen substances generated during the process can cause serious damage to neurons.[64] Due to microvascular thrombosis and microcirculation disturbance after SCI, vascular expansion, and blood flow restoration during reperfusion lead to the release of oxygen-free radicals into damaged sites, forming a toxic microenvironment for secondary nerve injury.[65–67] Although data on MSC-Exo in SCI is limited, anti-oxidative stress activities of MSC-Exo have been reported in many other diseases. It has been shown

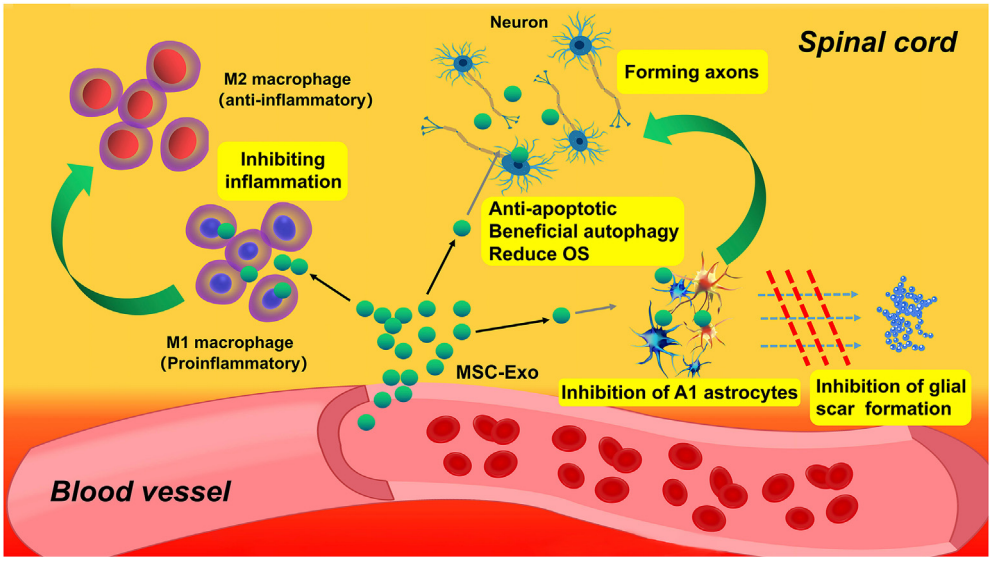

Fig. 3. Multitherapeutic effects of MSC-Exo in spinal cord injury recovery. M1 macrophages: classically activated macrophages; M2 macrophages: alternatively activated macrophages; OS: oxidative stress; MSC-Exo: exosomes derived from mesenchymal stem cells.

to remove harmful DNA fragments in the cytoplasm and circumvent oxidative stress-dependent DNA damage response to maintain intracellular homeostasis.[68] MSC-Exo can also reduce damage to renal epithelial cells caused by oxidative stress.[69] Shao et al.[70] ascertained that MSC-Exo significantly elevated H9C2 cell viability to counteract H2O2-induced apoptosis.

Superoxide dismutase (SOD) and reduced glutathione (GSH) have potent antioxidant effects, and their depletion may result in the accumulation of harmful oxygen-free radicals in the body.[71,72] Previous studies have proven that exosomes derived from induced pluripotent stem cells increased SOD and GSH expressions in the liver of rats, thereby reducing tissue damage caused by reactive oxygen species.[73] Yang et al.[74] found that MSC-Exo enhanced SOD and GSH activities, reducing malondialdehyde expression stimulated by oxidative stress and subsequent inflammatory mediator production.

MSC-Exo can aid in the persistence of balance between oxidative and antioxidant systems in response to noxious stimulation from SCI.

6.3. Anti-apoptotic effects: MSC-Exo inhibit neuronal apoptosis

Programmed cell death, or apoptosis, is initiated via activation of the apoptotic signaling pathway, which is another reason for neuronal loss after SCI.[75] Blocking apoptotic signaling pathways, together with stimulation of anti-apoptotic pathways, may rescue spinal cord neurons as much as possible.[4,76] The efficacy of exosomes in inhibiting neuronal apoptosis in SCI has been proven in animal experiments. Huang et al.[77] demonstrated a successful inhibition of neuronal apoptosis in SCI rats after systematic administration of MSC-Exo compared to untreated rats. MiRNA-abundant exosomes or MSC-Exo can elicit inhibitory effects on apoptosis signal transduction. Xiao et al.[78] utilized miR-134-abundant MSC-Exo to downregulate aspartic protease-8 activity to prevent oligodendrocyte apoptosis. Exosomal miR-21 has been shown to rescue apoptotic nucleus pulposus cells via PI3K/Akt activation.[79] MSC-Exo with miR-21-5p overexpression upregulated the FasL gene in spinal cord neurons in rats, reducing neuronal apoptosis, inflammation and promoting angiogenesis after SCI.[80]

In addition, exosomes capable of gene recombination reveal the therapeutic potential in neural repair. Luo et al.[81] transfected G protein-coupled receptor kinase 2 interacting protein 1 (Git1)-related gene-containing plasmid vectors into MSCs to obtain Git1-abundant exosomes, which inhibited cell apoptosis in spinal cord neurons, together with inhibited inflammatory response and glial scar formation, in SCI rats. Huang et al.[82] reported that miR-126-abundant exosomes significantly promoted vascular and nerve regeneration and suppressed neuronal apoptosis in SCI rats.

6.4. Regulation of autophagy: MSC-Exo reduce aberrant autophagy after SCI

Autophagy balances protein synthesis and degradation to preserve cell survival after tissue injury.[83,84] Physiologically, clearance of abnormally and excessively synthesized proteins or damaged organelles via lysosomes is beneficial to cell metabolism.[85] But persistent autophagy in response to SCI can lead to loss of neurons and neurological deficits.[86] Significant increases in the number of apoptotic cells and inflammatory factor expressions, including microtubule-associated protein light chain 3-II and Beclin 1, were observed in rats 8 h after SCI but were robustly reversed by 3-MA treatment 72 h after injury.[87] These studies indicate that autophagy is detrimental to healthy neurons after severe injuries like SCI.

Autophagic or apoptotic signalings or their components are involved in MSC-Exo-mediated autophagy regulation. Gu et al.[88] observed markedly increased expressions of the autophagy-related proteins light chain 3-IIB and Beclin-1 in SCI rats, as well as reduced neuronal apoptosis and improved functional behavior after MSC-Exo treatment. Fan et al.[89] found primary microglia-derived exosomes pretreated with resveratrol, an activator of autophagy, otherwise elicited a higher autophagic response, which is different from most findings, via PI3K signaling inhibition and accelerated recovery from lower limb paralysis in SCI rats. Ebrahim et al.[90] found that MSC-Exo-mediated autophagy inhibition was weakened by blocking the mechanistic target of rapamycin signaling. As current studies are confined to animal experiments and inconsistencies are present, the efficacy and feasibility of MSC-

Exo delivery in inhibiting excessive autophagy in SCI need to be validated in critically designed clinical trials.

6.5. Inhibition of reactive astrocytes: MSC-Exo thwart toxic astrocyte activation

Reactive astrocytes are of great significance in the repair of SCI, especially A1 astrocytes that have neurotoxic effects on neurons.[91,92] Increased proliferation in astrocytes mediated by NF-κB signaling activation can be observed after stimulation by the proinflammatory factors reactive oxygen species and IL-1, which trigger the secondary injury following SCI.[93,94] MSC-Exo can effectively inhibit the activation of A1 astrocytes to significantly improve the toxic environment surrounding the injured spinal cord in SCI rats.[95] Wang et al.[96] reported that MSC-Exo suppressed nuclear translocation of NF-κB p65, thus effectively reducing SCI-induced activation of A1 astrocytes. Overall, reactive astrocytes, particularly A1 astrocytes, are another therapeutic target for early intervention in SCI.

6.6. Promoting axon regeneration: MSC-Exo boost neurite outgrowth after SCI

The crux of neurofunctional recovery in SCI is axonal regrowth on the grounds that axons can be poorly self-regenerated in mature neurons, usually resulting in permanent neurological deficits in adult patients.[4,65,97] Successful axonal regeneration has been reported in experimental studies using miRNA-abundant MSC-Exo treatment.[98] The damaged area shrank pronouncedly, and longer axon lengths were measured in SCI rats treated with miR-126-modified MSC-Exo.[82] Besides, Li et al.[9] showed that exosomes containing miR-133b internally enhanced axon regeneration via boosting the signaling via extracellular regulated kinase 1/2-cyclic-AMP response binding protein and signal transducer and activator of transcription 3 signaling pathways.

Besides, in axon remodeling after SCI, glial scar at the damaged site is also a key obstacle to preventing axon growth.[3,99,100] MSC-Exo treatment against glial scar formation, a physical barrier, has been previously reported.[23,95,101]

The recent studies on MSC-Exo in the treatment of SCI are summarized in Table 1.

7. Natural advantages of exosomes as a drug carrier

7.1. Low immunogenicity enables exosomes to avoid immune exclusion

In general, traditional intravenous drugs are characterized by poor solubility, poor biocompatibility, low distribution of target tissue, and others, which are found as their defects.[102] Exosomes, as natural nanovesicles in organisms, consist of lipid bimolecular membrane and hydrophilic cavity. Exosomes outperforms traditional drug carriers in drug delivery, especially gene delivery.[36,103] Impacted by the specific proteins on the surface, the exosomes exhibit high stability in body fluids and are capable of avoiding the phagocytosis of the mononuclear phagocyte system; thus, exosomes do not lead to harmful immune exclusion.[104] In gene therapy, exosomes have a better affinity with nucleic acid molecules because of their hydrophilicity, thus significantly increasing the entrapment efficiency of inclusions; however, traditional liposomes have low entrapment efficiency for hydrophilic drugs and have certain limitations in nucleic acid delivery.[104,105]

7.2. Crossing the blood-brain barrier (BBB): exosomes can access the CNS

BBB consists of endothelial cells, astrocytes, and pericytes, which is a protective barrier required for the physiological activity of the CNS.[106] The above barriers are capable of preventing most small molecules and almost all macromolecules (e.g., polypeptides and nucleic acids) in the blood from entering the brain and spinal cord.[107] Existing studies confirmed that only 0.1% of peripherally administered antibodies are capable of crossing the BBB.[108] In contrast, exosomes are easier to cross the BBB due to their lipophilicity and small size (30 – 150 nm). Accordingly, exosomes, as a drug carrier, show promising applications in CNS diseases.

7.3. Imaging and tracing: exosomes can be easily visualized in vivo

The outer shell of exosomes has similarities to the cell membrane and can be exploited by lipophilic dyes (e.g., PKH67, PKH26, DiI, DiO, DiD, and DiR), in which the infrared fluorescence of DiR is capable of penetrating cells and tissues and has been generally used for tracing *in vivo* imaging.[109,110] Moreover, the unique vesicle structure can load a variety of tracer particles. For instance, the exosomes labeled with gold nanoparticles could be scanned using CT[111], and the exosomes labeled with superparamagnetic iron oxide nanoparticles could be tracked through simple MRI.[112] Furthermore, exosomes can display figures of exosomes distributed in the whole body of organisms based on bioluminescence imaging.[113] In brief, exosomes can be traced using different tracing methods, and *in vivo* tracing can present the biological distribution, migration ability, and communication mechanism of exosomes.

7.4. MSC-Exo as drug carrier to treat SCI

As exosome research has been leaping forward, exosomes not only contain a wide range of biomolecules for treating SCI but also have served as a drug carrier to treat SCI.

8. Comparing exosomes from different stem cell sources for SCI treatment

Emerging evidence suggests that the source of stem cells used to derive exosomes can influence their therapeutic effects for SCI treatment. For instance, exosomes isolated from BMMSCs overexpressing miR-137 were found to reduce apoptosis and inflammation while promoting angiogenesis and functional recovery in a rat contusion SCI model.[114] This was attributed to miR-137 regulating downstream phosphatase and tensin homolog (PTEN)/AKT signaling. In comparison, exosomes derived from human umbilical cord MSCs could attenuate neuroinflammation and improve locomotor function in mice after SCI, partially by deactivating microglia/macrophages.[7] The unique umbilical origin of human umbilical cord MSCs may confer enhanced anti-inflammatory effects on their exosomes. Meanwhile, Jafari et al.[115] reported that exosomes from human placenta-derived MSCs worked synergistically with hyperbaric oxygen therapy to mitigate oxidative stress, apoptosis, and mitochondrial dysfunction in a spinal cord ischemia-reperfusion injury rat model. This indicates the antioxidant capacities of human placental MSC-Exo, likely due to placental MSCs residing in a hypoxic niche. Intriguingly, neural stem cell (NSC) derived exosomes have recently exhibited neuro regenerative potential for SCI by transferring microRNAs like miR-133b to stimulate neurite remodeling and axon growth.[116] The intrinsic neurotropism of NSCs

Table 1
Summary of recent studies on MSC-Exo in the treatment of SCI.

Study	Year	Type of MSCS	Modifier	Animals	Results	Brief mechanism
Lu et al.[6]	2019	BMMSCs	None	Rats	Reduce pericyte migration, thereby improving the integrity of the blood-spinal cord barrier.	Restore the integrity of the blood-spinal cord barrier.
Sun et al.[7]	2018	hucMSCs	None	Mice	Trigger the polarization of bone marrow-derived macrophages from M1 to M2 phenotype. Down regulation of TNF-α, MIP-1 α, IL-6 and IFN- γ.	Inhibit inflammation
Chang et al.[8]	2021	BMMSCs	None	Rats	Promote phenotypic polarization of large M2 macrophages. Inhibit neuronal apoptosis, degeneration, and inflammatory response induced by SCI. The decrease of IRF5 expression inhibits the differentiation of macrophages into M1 phenotype and the secretion of inflammatory factors.	Inhibit inflammation; anti-apoptotic
Li et al.[9]	2018	BMMSCs	miR-133b	Rats	Reduce the volume of injury, preserve neuronal cells, and promote the regeneration of axons after spinal cord injury. MiR-133b activates ERK1/2, STAT3, and CREB signaling pathways and is a signaling pathway protein involved in neuronal survival and axon regeneration.	Anti-apoptotic; ERK1/2, STAT3, and CREB signaling pathways promote neuronal survival and axon regeneration.
Massoto et al.[11]	2018	BMMSCs	Treadmill training	Mice	The area of spare white matter is larger and the number of myelinated fibers retained is greater. The tissue was well preserved, with more micro vessels and less nerve fiber degeneration. Significantly higher NT4 expression.	Anti-apoptotic; more neurotrophic factors
Zhao et al.[12]	2019	BMMSCs	None	Rats	Decreased SCI-induced complement levels. Inhibited SCI-activated NF- κB.	Inhibiting complement mRNA synthesis and release and inhibiting SCI-activated NF-κB by binding to microglia.
Zhou et al.[13]	2021	hpMSCs	None	Rats	Promote the activation of proliferating endogenous neural stem/progenitor cells. There was a significant increase in SOX2+GFAP+, PAX6+Nestin+, and SOX1+KI67+ cells in the spinal cord. Higher neurogenesis, as shown by a higher percentage of DCX + map 2 + neurons. The proliferation of neural stem cells and the increase of MEK, ERK, and CREB}? phosphorylation levels.	Activation of endogenous neural stem cells.
Huang et al.[23]	2021	BMMSCs	CTGF-siRNA	Rats	Inhibit CTGF gene, thus inhibiting inflammation, and preventing neuronal apoptosis and reactive astrocyte and glial scar formation. Upregulation of several neurotrophic factors and anti-inflammatory factors.	Inhibit CTGF gene; inhibit inflammation; anti-apoptotic; inhibit the activation of reactive astrocytes.
Zhang et al.[24]	2020	hpMSC	None	Rats	Tube formation and migration of HUVECs. The number of blood vessels, vascular volume fraction, and vascular connectivity in the spinal cord increased significantly.	Tube formation and migration of HUVECs
Guo et al.[27]	2019	BMMSCs	PTEN-siRNA	Rats	Enhanced axon growth and neovascularization. Reduce microglia proliferation and astrocyte proliferation. Improve neural structure and electrophysiological function.	Decrease the expression of PTEN in the injured spinal cord; inhibit inflammation; anti-apoptotic; inhibit the activation of reactive astrocytes; axon regeneration
Li et al.[31]	2020	hMSCs	Adhesive Hydrogel	Rats	Comprehensively alleviate SCI microenvironment. Effective retention and sustained release of exosomes in the host. Effectively reduce inflammation and oxidation.	Inhibit inflammation; reduce oxidative stress; drug retention and slow release
Kim et al.[32]	2018	hMSCs	IONP	Rats	The magnetic conductivity of IONP. Carry more therapeutic growth factors. Iron oxide nanoparticles activate JNK and c-Jun signal cascades in hMSCs. Promote the angiogenesis of injured spinal cord and reduce inflammation and neuronal apoptosis.	Drug-targeted enrichment; Inhibit inflammation; More neurotrophic factors; Anti-apoptotic; JNK and c-Jun signal; axon regeneration
Ji et al.[37]	2019	BMMSCs	None	Rats	The exosomes of MSC in obese rats showed a decreased level of miR-21 and had no protective effect on SCI.	The lack of miR-21 in MSCs of obese rats leads to the effect of MSCs-Exo of obese rats on SCI.
Li et al.[54]	2020	BMMSCs	miR-544	Rats	Reduce inflammation after SCI. Promote neuronal survival.	Inhibit inflammation; anti-apoptotic
Fan et al.[55]	2021	BMMSCs	None	Rats	Inhibition of neuronal apoptosis. Reduced the secretion of pro-inflammatory factors including TNF-α and IL-1β and promoted the secretion of anti-inflammatory factors including IL-10 and IL-4.	Inhibit inflammation; anti-apoptotic; inhibiting the TLR4/MyD88/NF-κB signaling pathway
Liu et al.[60]	2020	BMMSCs	miR-216a-5p	Mice	Microglia polarization was transformed from the M1 phenotype to the M2 phenotype. miR-216a-5p may be involved in microglia polarization. TLR4/NF-κB/PI3K/AKT signal cascade may be involved in the regulation of microglia polarization.	Inhibit inflammation; miR-216a-5p mediates TLR4/NF-κB/PI3K/AKT signaling cascade promotes the transformation of microglia M1 phenotype to M2 phenotype.
Li et al.[62]	2020	BMMSCs	None	Rats	Enhanced M2 polarization. Block apoptosis. Reduce SCI-induced tissue injury and nerve injury.	Inhibit inflammation; anti-apoptotic; MiR-124-3p in exosomes reduces SCI-induced nerve injury by regulating the polarization of ern1 and M2 macrophages.

(*continued on next page*)

Table 1 (*continued*)

Study	Year	Type of MSCS	Modifier	Animals	Results	Brief mechanism
Li et al.[69]	2019	BMMSCs	None	Rats	Inhibit neuronal apoptosis. Promote the expression of Bcl-2 and inhibit the expression of Bax, cleaved caspase-3 and cleaved caspase-9.	Anti-apoptotic; active Wnt/β-Catenin signal pathway.
Huang et al.[77]	2017	BMMSCs	None	Rats	Reduce apoptosis and inflammation of the injured spinal cord. Reduce the expression of Pro apoptotic proteins (Bcl-2) and pro-inflammatory cytokines (TNF- α and IL-1β). Promote the expression of anti-apoptotic proteins (Bcl-2) and anti-inflammatory proteins (IL-10). Promote angiogenesis.	Inhibit inflammation; anti-apoptotic
Xiao et al.[78]	2019	BMMSCs	None	Rats	MiR-134 inhibits apoptosis by reducing the expression of caspase-8.	Anti-apoptotic
Zhou et al.[80]	2019	BMMSCs	None	Rats	Reduce apoptosis, inflammation and promote angiogenesis. Reduced lesion size and apoptosis. Inhibition of miR-21-5p in MSCs-EVs can significantly improve motor function and apoptosis.	Inhibit inflammation; Anti-apoptotic; miR-21-5p/FasL gene axis
Luo et al.[81]	2021	BMMSCs	GIT1	Rats	Inhibit the formation of glial scar and neuroinflammation. Inhibited apoptosis in the injured area. Promotes axon regeneration.	Inhibit inflammation; anti-apoptotic; axon regeneration
Jhha et al.[82]	2020	BMMSCs	miR-126	Rats	Reduce damage volume. Promotes angiogenesis after SCI. Promotes neurogenesis and reduces apoptosis after SCI.	Inhibit inflammation; anti-apoptotic; axon regeneration; miR-126 promotes angiogenesis and neurogenesis by inhibiting the expression of SPRED1 and PIK3R2.
Gu et al.[88]	2020	BMMSCs	None	Rats	Promote the expression of autophagy-related proteins LC3IIB and Beclin-1. Promote the formation of autophagosomes. Decreased Pro apoptotic protein (caspase-3). Promote the upregulation of anti-apoptotic protein (Bcl-2).	Anti-apoptotic; the formation of autophagosomes.
Liu et al.[95]	2019	BMMSCs	None	Rats	Promote angiogenesis. Attenuate neuronal apoptosis and glial scar formation, Inhibit inflammation. Promote axon regeneration. Inhibit the activation of A1 neurotoxic reactive astrocytes.	Inhibit inflammation; anti-apoptotic; inhibit the activation of reactive astrocytes
Wang et al.[96]	2018	BMMSCs	None	Rats	Reduce the proportion of A1 astrocytes induced by SCI. Reduce the expression of IL-1α and IL-1β. Promote the expression of MBP, Syn, and NeuN.	Inhibit inflammation; axon regeneration; Inhibit SCI-induced activation of A1 astrocytes by inhibiting the nuclear translocation of the NF-κB p65.
Yu et al.[98]	2019	BMMSCs	miR-29b	Rats	NF200+ and GAP-43+ neurons increased. The number of contractile nerve cells and GFAP + neurons decreased.	Anti-apoptotic; axon regeneration

MSC-Exo: exosomes derived from mesenchymal stem cells; SCI: spinal cord injury; MSCs: mesenchymal stem cells; BMMSCs: bone marrow mesenchymal stem cells; hucMSCs: human umbilical cord mesenchymal stem cells; TNF-a: tumor necrosis factor-a; IFN-g: interferon-g; IRF5: interferon regulatory factor 5; MIP-1: macrophage inflammatory protein 1, IL: interleukin; MiR-133b: microRNA-133b; ERK1/2: signaling via extracellular regulated kinase 1/2; STAT3: signal transducer and activator of transcription 3; CREB: cyclic-AMP response element-binding protein; NT4: neurotrophin 4; NF-kB: nuclear factor kappa-B; mRNA: messenger RNA; hpMSC: human placental mesenchymal stem cells; SOX2: SRY-box transcription factor 2; GFAP: glial fibrillary acidic protein; PAX6: paired box protein 6; DCX: doublecortin; MEK: methyl ethyl ketone; ERK: extracellular signal-regulated kinase; CTGF: connective tissue growth factor; SiRNA: small interfering RNA; HUVECs: human umbilical vein endothelial cells; PTEN: phosphatase and tensin homolog; hMSCs: human mesenchymal stem cells; IONP: Iron oxide nanoparticle; JNK: c-Jun N-terminal kinase; miR: microRNA; TLR4: toll-like receptor 4; PI3K: phospoinositide 3-kinases; AKT: protein kinase B; Bcl-2: B-cell lymphoma-2; EVs: extracellular vesicle; GIT1: G-protein-coupled receptor kinase interactor-1; SPRED1: sprouty related EVH1 domain containing 1; PIK3R2: phosphoinositide-3-kinase regulatory subunit 2; LC3-IIB, light chain 3-IIB; MBP: Myelin basic protein; Syn: synaptophysin; NeuN: neuronal nuclei; NF200: neurofilament-200; GAP-43: growth associated protein-43.

may allow their exosomes to directly activate neuronal regenerative programs after SCI.

In summary, while MSC-Exo from various tissue sources share common anti-inflammatory and neuroprotective properties, exosomes retain some intrinsic therapeutic characteristics based on the origin of parent stem cells. In particular, NSC-derived exosomes may confer unique pro-regenerative effects. Systematically comparing exosome bioactivity and RNA/protein cargo from diverse stem cell types could elucidate optimal exosome sources for combination therapy. This may involve co-administering NSC-derived exosomes to promote axon regeneration along with MSC-Exo to modulate inflammation. Further research into the functional outcomes of different stem cell-derived exosomes will help advance personalized, precision exosome therapies for SCI.

9. Loading therapeutic contents into exosomes: RNAs, proteins, chemicals

9.1. RNAs drugs

Exosomes are natural RNA carrier, capable of preventing RNA from being degraded by different ribonucleases *in vivo*.[103] In general, RNAs drugs consist of miRNAs, siRNA, and long noncoding RNAs. The above RNAs are noncoding RNA and cannot be translated into proteins, whereas they have been found to play a vital role in controlling the biological activities of the body.[51,103] To be specific, miRNAs are most extensively used. MiRNAs have been reported to play an essential role in various stages of neural development, injury, and progression of neurological diseases.[60,98] For instance,

Liu et al.[117] delivered miR-455-5p using MSC-Exo, which could inhibit neuronal apoptosis and activate neuronal autophagy in SCI rats. Huang et al.[82] delivered miR-126 to target the RhoA pathway using MSC-Exo, to facilitate axon regeneration and reduce pathological damage in SCI rats. Besides, RNA interference technology has been reported to be effective in downregulating the expressions of harmful genes in the SCI process. In our previous study, the siRNA silencing connective tissue growth factor (CTGF) gene was also loaded through MSC-Exo, thus facilitating the recovery of motor function in SCI rats.[23] Guo et al.[27] repaired complete SCI with MSC-Exo containing phosphatase and tensin homolog-siRNA through nasal administration, and a good therapeutic effect was achieved. Besides, De Rivero et al.[118] employed neuron-derived exosomes to load siRNA targeting the caspase recruitment domain, which could down regulate the level of caspase recruitment domain protein in SCI rats by 76%.

Long noncoding RNA (lncRNA) refers to a non-coding RNA with transcripts longer than 200 nt, playing a certain role in the repair of SCI after various stages of epigenetic regulation, transcriptional regulation, and post-transcriptional regulation.[119,120] As reported by Liu et al.[121], MSC-Exo complexed with lncRNA-TCTN2 could regulate the miR-329-3p/IGF1R axis and facilitate the repair of SCI. Moreover, Shao et al.[122] reported that adipose tissue-derived mesenchymal stem/stromal cells derived exosomes loaded with lncRNA-Gm37494 could repair SCI by regulating the M1/M2 polarization of microglia.

9.2. Protein drugs

Furthermore, proteins are also vital biological macromolecules regulating the repair of SCI (e.g., ubiquitin thioesterase outline, advances in human genetics have identified angiopoietin-like 3, and nerve growth factor), which have been confirmed to play an active role in the pathophysiological process after SCI.[123–125] Luo et al.[123] confirmed that macrophage-derived, exosomes loaded with ubiquitin thioesterase outline could stimulate Wnt/β-catenin signaling mediated vascular regeneration to facilitate the recovery of SCI. Moreover, as reported by Cao et al.[124], local delivery of human urine stem cell-derived exosomes containing advances in human genetics has identified angiopoietin-like 3 facilitated functional recovery after SCI through the promotion of angiogenesis. In addition, Chen et al.[126] suggested that neural stem cell-derived exosomes loaded with FTY720 facilitated the recovery after SCI in rats by inhibiting the PTEN/AKT signaling pathway.

9.3. Traditional chemical drugs

Although traditional chemical drugs have been reported to play a certain role in treating SCI, they are poorly absorbed *in vivo* for their low water solubility, low solubility, poor specificity, and significant side effects.[104] Interestingly, exosomes are capable of avoiding the above defects. As reported by existing studies, resveratrol-induced exosomes are capable of inhibiting apoptosis by activating autophagy and PI3K signaling pathways, thus facilitating the recovery of motor function in SCI rats.[89] According to Zhang et al.[127], primary M2 macrophage-derived exosomes loaded with nerve growth factor and curcumin could facilitate the repair of SCI based on their anti-inflammatory and neuroprotective properties. Gao et al.[128] verified that berberine-loaded M2 macrophage-derived exosomes could enhance the inflammatory response after SCI. In brief, the above studies reported that exosomes also play a fabulous role in the delivery of traditional chemical drugs.

10. Methods for loading drugs into exosomes

At present, based on whether the drug is directly loaded on exosomes, the types of drug loading modes largely fall into pre-secretory loading and post-secretory loading.[47] Pre-secretory loading refers to the direct loading/transfection of drugs on the secretory cell to secrete drug-loaded exosomes, i.e., direct incubation.[104] The above operations are simple, whereas the drug loading efficiency is low and likely to disrupt the natural physiological function of membrane proteins.[104] Moreover, post-secretory loading is known as the direct addition of drugs to exosomes in a certain method, thus significantly increasing the load amount of exosomes. To be specific, electroporation, chemical transfection, and ultrasonication are the most common post-secretory loading methods.[104,129]

10.1. Direct incubation

Direct incubation aims to co-incubated the drug with the parental cells, load part of the drug in the cytoplasm into the secreted exosomes through the endocytosis of the parental cells, or directly mix and co-incubated the exosomes and the drug under appropriate conditions.[104] The above 2 methods exhibit the advantages of simple operation, strong reproducibility, and no effect on the integrity of exosome membrane structure, whereas they are characterized by low loading efficiency and require considerable exosomes and drugs.[104] Luo et al.[81] employed adenoviruses to transfect Git1 into BMMSCs. As a result, exosome-Git1 with the effect of treating SCI was extracted after 48 h of incubation.

10.2. Electroporation

Electroporation has been recognized as a relatively mature transfection method, which exploits a brief high-voltage pulse to instantly reduce the permeability of the exosome membrane, thus opening a temporary pore for the drug to penetrate the exosome.[104] As early as 2013, researchers transfected anti-RAD51 siRNA into exosomes through electroporation and cultured the therapeutic exosomes with recipient cancer cells, which led to considerable cancer cell death.[130] Huang et al.[23] loaded anti-CTGF siRNA into MSC-Exo through electroporation and constructed exosome-siRNA, thus facilitating nerve regeneration and functional recovery in SCI rats *in vivo* and *in vitro*. Moreover, Li et al.[54] successfully transfected miR-544 mimic into MSCs using Lipofectamine 3000, and the exosomes containing miR-544 were extracted and successfully applied for the repair and treatment of SCI. In general, electroporation is simple and time-saving, and it is primarily adopted to load nucleotides into exosomes, whereas its loading efficiency is low, usually about 30%. Besides, high-voltage pulses may damage surface protein structures and affect exosome activity.

10.3. Chemical transfection

Benefiting from the rapid development of exosome drug delivery technology, some companies have developed a specialized Exosome Transfection Kit (Exo-fect), capable of directly loading si/miRNA, mRNA, and even plasmid DNA into exosomes; this kit is characterized by a short loading time (usually less than 15 min) and high loading efficiency (up to 95%).[131,132] Moreover, the Exo-fect reagents are not as toxic as lipid-based transfection reagents. According to our previous study, miR-494 was successfully transfected into BMMSC-Exo with Exo-fect to construct ExomiR-494. Subsequently, animal studies demonstrated that ExomiR-494 can effectively release miR-494 to the SCI area and upregulate the local

concentration of miR-494, thus inhibiting the inflammatory response and protecting neurons.[133]

10.4. Ultrasonication

Ultrasonication has been rarely utilized thus far, whereas this method has high drug loading efficiency and strong drug release ability and can prevent the destruction of blood enzymes.[134] However, it may cause exosome aggregation to affect their immune activity. Moreover, the loading process is likely to disrupt the plasma membrane structure of exosomes and cause drug leakage.[135]

11. Engineering exosomes to improve drug delivery in SCI

Even though exosomes have numerous advantages, natural exosomes are characterized by low yield, low targeting, poor stability, fast degradation, and others, thus reducing the drug load and the targeting effect on organs/cells.[36] Accordingly, the modification of exosomes to facilitate the production of exosomes, drug loading, and targeting has also been the focus of exosome drug loading research. Over the past few years, commonly used methods have consisted of click chemistry, genetic engineering, magnetic nanoparticle technology, electrostatic interaction, etc. For instance, Haraszti et al.[136] adopted the microcarrier-based 3-dimensional (3D) cultures to make umbilical cord-derived MSCs produce exosomes 20 times more than that produced by the 2-dimensional (2D) cultures. Kim et al.[32] also introduced IONP into MSC-derived nanovesicles and subsequently used magnetic navigation to deliver the modified nanovesicles to the central area of SCI, which significantly increased the concentration of local drugs; besides, exogenous IONP also stimulated the secretion of various neurotrophic factors, thus doubly increasing the therapeutic effect of SCI. Besides, some researchers combined engineering technology to immobilize exosomes in peptide-modified 3D adhesive hydrogel and transplant them into SCI rats. As a result, the hydrogels facilitated the sustained release and uniform distribution of exosomes.[31] Furthermore, Zhang et al.[137] combined novel bio-specificity peptides and exosomes to tether therapeutic exosomes on collagen scaffolds to enhance local drug concentration.

In brief, the above methods of modification or engineering of exosomes increased the yield of exosomes and the enrichment concentration at the damaged site to a certain extent, thus maximizing their drug-carrying effect as a drug-carrying tool (Fig. 4).

While MSC-Exo have demonstrated therapeutic efficacy for SCI treatment, recent advances reveal that exosomes derived from diverse stem cell sources have unique properties that may be leveraged for multifaceted repair strategies. Neural stem cells derived exosomes enriched with miR-133b were found to enhance neurite remodeling and functional recovery in SCI rodent models, attributed to miR-133b's role in regulating axon guidance and neurite outgrowth genes.[9] The inherent neurotropic effects of neural stem cell-exosomes highlight their potential to directly stimulate axonal regeneration. Meanwhile, adipose-derived stem cell exosomes containing miR-21-5p could suppress apoptosis, alleviate inflammation, and promote angiogenesis following SCI, partially by modulating downstream miR-21-5p gene targets like programmed cell death 4 and FasL.[138] The anti-apoptotic and pro-angiogenic capacity of adipose stem cell-exosomes may confer neuroprotective and vascular benefits.

In comparison, MSC-Exo possess unique immunomodulatory properties, as they can reprogram macrophages from cytotoxic M1 to anti-inflammatory M2 phenotypes and downregulate pro-inflammatory cytokines through exosomal transfer of regulatory miRNAs.[139]

12. Limitations of MSC-Exo for SCI treatment

However, MSC-Exo may have limited intrinsic neurotrophic effects. Comparative analysis of stem cells derived exosomes reveals that each type has complementary therapeutic mechanisms based on the native functions of their parent cells. Combining exosomes from multiple sources could allow for targeting diverse secondary injury pathways underlying SCI pathology. For instance, co-administering adipose stem cell-derived exosomes to enhance angiogenesis with MSC-Exo for immunomodulation may elicit synergistic benefits. Further elucidation of the bioactive cargo in different exosome populations could uncover optimal combinations for stem cell-free SCI therapy.

While MSC-Exo have demonstrated therapeutic promise for SCI, optimizing their targeted delivery to injury sites remains a key challenge. Systemically administered exosomes show low targeting efficiency and rapid clearance. One approach is engineering exosomes to display targeting peptides or antibodies on their surface to enable binding to receptors upregulated after SCI, like vascular cell adhesion molecule 1 or matrix metalloproteinases.[140] Loading exosomes into biomaterial scaffolds implanted at the lesion site could also allow sustained, localized delivery. However, most targeting strategies remain limited to preclinical testing.

Additionally, several limitations currently hinder the clinical translation of MSC-Exo therapy for SCI. A major constraint is the poor scalability of exosome production, which faces challenges of inefficient isolation yields, heterogeneity between preparations, and high costs.[141] Variability in exosome bioactivity based on different MSC sources and culture conditions also needs to be addressed. Moreover, while MSC-Exo confers neuroprotection, they may have a restricted capacity to directly stimulate axon regeneration across the glial scar. Combining MSC-Exo with exosomes engineered for enhanced neurite outgrowth could help overcome this limitation. Another concern is the short half-life and rapid clearance of systemically delivered exosomes. Developing controlled-release formulations or local delivery strategies may prolong their therapeutic availability. Further research tackling these limitations is imperative to facilitate the transition of MSC-Exo therapy from proof-of-concept to clinical implementation for SCI treatment.

13. Combining regenerative medicine to treat SCI

In parallel with advances in exosome technology, breakthroughs in biomaterials and tissue engineering have enabled novel hybrid strategies for SCI treatment. Implanting conductive biomaterial scaffolds in SCI lesions can provide a growth-permissive microenvironment for regenerating axons while also restoring lost tissue.[139] Scaffolds derived from natural biopolymers or synthetic hydrogels can be tuned to mimic the stiffness, topography, and electrical conductivity of the native spinal cord extracellular matrix to guide axon growth.[142] Moreover, scaffolds embedded with controlled release systems for anti-inflammatory drugs, neurotrophic factors, or exosome payloads can act as bioactive platforms to modulate the cellular response. Recent clinical trials indicate that implanting scaffold-neural progenitor cell constructs after SCI can promote modest sensory and motor improvements, providing a foundation for further optimization.[142]

An exciting prospect is synergistically combining scaffold implants with stem cell and exosome therapies for multi-pronged SCI repair. For instance, conductive scaffolds loaded with MSC-Exo could simultaneously provide biochemical support through immunomodulation while enabling structural guidance of regenerating axons across the lesion site.[138] Scaffolds may also allow for controlled, localized delivery of exosomes to enhance treatment

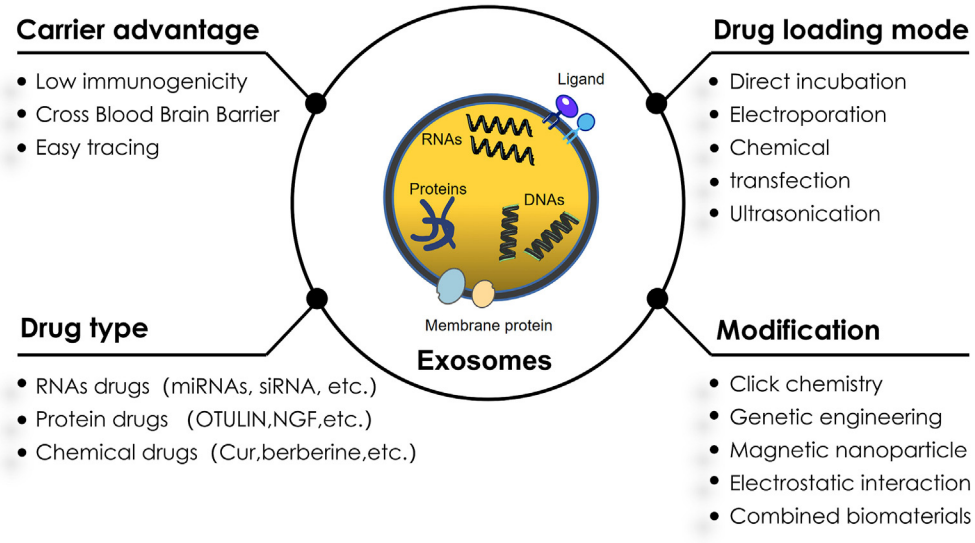

Fig. 4. Strategy of exosomes as drug carriers in the treatment of spinal cord injury.

efficiency compared to systemic injection. Moving forward, integrated biomaterial-cell-exosome approaches offer immense potential for overcoming the intrinsic limits to functional recovery after severe SCI. Realizing the full regenerative capacity of these bioengineered therapies will require iterative evaluation in preclinical animal models followed by careful clinical translation. Nevertheless, the convergence of materials science, stem cell biology, and exosome technology promises new hope for restoring spinal cord connectivity and mobility after devastating injury.

14. Summary and perspective

An original characteristic of this review is the roles of MSC-Exo in controlling secondary nerve injury following SCI, which gives rise to a new tool to minimize damaged areas and promote nerve remodeling and offers new hope for a better prognosis. Current experiments focus on but will not be confined to MSC-Exo transfected with single genes or molecules given the various roles of MSC-Exo in inhibiting the inflammatory response, oxidative stress, neuronal apoptosis, and toxic astrocyte activation and promoting beneficial autophagy and axon regeneration in SCI (Fig. 3). Moreover, as a drug loading tool, exosomes have a variety of natural advantages, such as low immunogenicity, strong tissue permeability, and homing benefits. However, exosomes also have the defects of low yield and insufficient targeting. Therefore, it is a necessary way to modify exosomes and explore effective engineering exosomes (Fig. 4). Furthermore, accurate mechanisms for intercellular exchange of information between exosomes and target cells have not been fully clarified, which may aid in the identification of potential therapeutic targets for MSC-Exo treatment. The feasibility, safety, and efficacy of exosomes for SCI treatment needs to be critically proved by randomized controlled trials.

While exosomes represent a promising therapeutic approach for SCI, scaling up production and ensuring manufacturing quality control remain key obstacles for clinical translation. Isolating clinical-grade exosomes faces challenges of limited cell source availability, variability in isolation yields, and high costs of current methods like ultracentrifugation. Advances in bioreactor cultures using microcarriers or hollow-fiber membranes could enable large-scale expansion of exosome-producing stem cells. Further optimization of scalable isolation methods like tangential flow filtration and sterile purification protocols will be critical. Moreover, techniques to characterize the bioactive molecular cargo of exosome batches and standardize potency assays need to be established. Addressing these production and quality control issues through integrated bioprocessing strategies will accelerate the journey of exosome therapies from bench to bedside. The advent of synergistic biomaterial-cell-exosome approaches may also help overcome delivery limitations and enhance localized treatment while minimizing required doses. Continued multidisciplinary collaboration tackling in these manufacturing challenges will enable the full clinical potential of exosome therapeutics to be realized.

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Ethical statement

Not applicable.

Declaration of competing interest

The authors declare that they have no competing interests.

Author contributions

Lin-Fei Cheng is in charge of experimental design; Chao-Qun You and Cheng Peng are responsible for data statistics; Jia-Ji Ren and Kai Guo are responsible for data analysis; Tie-Long Liu is responsible for experimental guidance; All authors provided intellectual input and edited and approved the final manuscript.

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